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(74) Agent: PASSOWICZ, Marek; Dr. A.Au & Co., ul. skiego 27/29, P.O. Box 85, PL-60-967 Poznań (Pl		-

(54) Title: ANTICANCER VACCINE COMPRISING IL6/IL6 RECEPTOR TRANSFECTED CELLS

(57) Abstract

Genetic anticancer vaccine for stimulation of patient's immune system to eradicate cancer, particularly malignant melanoma. The objective of the invention is genetic modification of allogeneic cancer cells by insertion of the two genes, one encoding human interleukin 6 and the other encoding soluble interleukin 6 receptor, which will be administered to patients.

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ANTICANCER VACCINE COMPRISING IL6/IL6 RECEPTOR TRANSFECTED CELLS

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The objective of the invention is genetic anticancer vaccine for gene therapy of human neoplastic diseases particularly malignant melanoma.

Concept of so called genetic cellular vaccines is based on genetic modification of autologous (patient's own) or antigenetically related (allogeneic) cancer cells in order to activate patient's immunologic system to eliminate cancer. Autologous (obtained from each patient to be treated) and/or allogeneic (established cancer cell lines) genetically modified cancer cells are irradiated and injected subcutaneously to the patient. Until now cancer (autologous or allogeneic) cells in order to provide costimulatory signal for patient's own immune system have been genetically modified by insertion of various genes encoding: interleukin (IL) 2 [allogeneic cells; (1)], IL-4 (2), IL-7 (3), tumor necrosis factor [TNF; (4)], interferon gamma (5) or macrophage-granulocyte colony stimulating factor [GM-CSF) (6)] (autologous cells).

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The objective of the invention is genetic modification of human malignant melanoma cell line which is HLA-A1 and HLA-A2 positive by introduction into the cells two genes (cDNA) coding human IL-6 and soluble IL-6 receptor (sIL-6R).

SIL-6R was constructed by replacement of cytoplasmic and transmembrane domains of the membrane receptor by translational stop codon using polymerase chain reaction (PCR) with primer 5'CGGATCCGTCGACTAATCTTGGCACTGGGAGGCTTG3'. Moreover, signal peptide was replaced by translational start codon ATG using PCR with primer 5'GGGGACATGTTAGCCCCAAGGCGCTGCCCT3', introducing methionine as a first aminoacid of sIL-6R.

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Another objective of the invention is a vaccine containing autologous cancer cells and genetically modified allogeneic cancer cells. Combination of autologous and allogeneic cells will increase immunogenicity and effectiveness of the vaccine. In this variant of the vaccine autologous cells do not require genetic modification. Products of introduced genes will be supplied by allogeneic cells and their biological effect will be provided by "by stander effect".

EXAMPLES OF APPLICATION OF THE INVENTION

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- 1. From the melanoma patient (HLA-A1 and/or HLA-A2 positive) a cancer metastatic focus will be surgically excised. Obtained tissue will be minced, cells enzymatically isolated and either frozen in liquid nitrogen or cultured in vitro in typical conditions. After obtaining in culture required number of cells they will be mixed (1 : 1) with genetically modified allogeneic cells. If propagation of autologous cells in vitro will not be possible cells frozen in liquid nitrogen will be thawed and used. Then the mixture (5 x 10⁷ cells per injection) will be irradiated using a total dose of 100 Gy and subcutanousely administered to the patient. Four injections will be administered in two weeks intervals followed by three injections once a month. If necessary injections will be continued in two months intervals.
- 2. In some melanoma patients excision of metastases will not be possible due to the advancement of the disease or localization of lesions. In such cases allogeneic vaccine will be applied. Genetically modified cells (5×10^7) will be irradiated and administered as described above.

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CLAIMS

- 1. Genetic anticancer vaccine, containing genetically modified allogeneic cancer cells, characterized in, that allogeneic cells contain two genes, one encoding interleukin 6 (IL-6), and the other encoding interleukin 6 soluble receptor (sIL-6R).
 - 2. Genetic anticancer vaccine according to claim 1. characterized in, that contains gen (cDNA) for human IL-6 and gen (cDNA) for human sIL-6R, while sIL-6R is a modified membrane receptor in which cytoplasmic and transmembrane domains were replaced by translational stop codon, and signaling peptide was replaced by translational start codon.
 - 3. Genetic anticancer vaccine, containing autologous cancer cells, characterized in, that it also contains genetically modified allogeneic cancer cells, while content of allogeneic cells can not be lower then 50% and can not exceed 70%.

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A. CLASSIFICATION OF SUBJECT MATTER IPC 6 A61K39/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols) IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

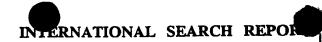
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Date of the actual completion of the international search 17 January 1997	Date of mailing of the international search report 2 9. 01. 97
Name and mailing address of the ISA	Authorized officer

Sitch, W

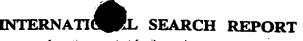
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